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# FEE TRANSMITTAL For FY 2005

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ ) 2,540.00

## Complete if Known

Application Number 09/825,769  
Filing Date April 4, 2001  
First Named Inventor Milan S. BLAKE et al.  
Examiner Name V. Ford  
Art Unit 1645  
Attorney Docket No. 38777-0054

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## FEE CALCULATION

### 1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES		SEARCH FEES		EXAMINATION FEES		Fees Paid (\$)
	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	Fee (\$)	Small Entity Fee (\$)	
Utility	300	150	500	250	200	100	
Design	200	100	100	50	130	65	
Plant	200	100	300	150	160	80	
Reissue	300	150	500	250	600	300	
Provisional	200	100	0	0	0	0	

### 2. EXCESS CLAIM FEES

Fee Description	Fee (\$)	Small Entity Fee (\$)
Each claim over 20 or, for Reissues, each claim over 20 and more than in the original patent	50	25
Each independent claim over 3 or, for Reissues, each independent claim more than in the original patent	200	100
Multiple dependent claims	360	180

**Total Claims**          **Extra Claims**          **Fee (\$)**          **Fee Paid (\$)**           
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 HP = highest number of total claims paid for, if greater than 20  
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Other: Appeal Brief \$500/Petition for Extension of Time (2-5) \$2040

**Fees Paid (\$)**

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## SUBMITTED BY

Signature John P. Isacson Registration No. (Attorney/Agent) 33,715 Telephone (202) 912-2000  
Name (Print/Type) John P. Isacson Customer No. 26633 Date December 14, 2004

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Attorney Docket No.: 38777-0054

Applicant(s): Milan S. BLAKE *et al.*

Confirmation No.: 3657

Appl. No.: 09/825,769

Art Unit: 1645

Filing Date: April 4, 2001

Examiner: Vanessa L. Ford

Title: METHOD FOR THE PRODUCTION OF BACTERIAL TOXINS

Commissioner for Patents  
Mail Stop Appeal Brief - Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**APPEAL BRIEF**

John P. Isacson  
Heller Ehrman White & McAuliffe LLP  
1666 K Street, N.W., Suite 300  
Washington, D.C. 20006  
Telephone: (202) 912-2000  
Facsimile: (202) 912-2020  
Customer No. 26633

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
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Examiner: Vanessa L. Ford

Title: METHOD FOR THE PRODUCTION OF BACTERIAL TOXINS

**APPEAL BRIEF**

Commissioner for Patents  
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Alexandria, VA 22313-1450

Further to the Notice of Appeal filed on May 17, 2004, Appellants file this Appeal Brief. Appellants petition for a extension of time for the second, third, fourth and fifth months, as well as any other needed extension, and provide the requisite fee herewith. The first month extension was paid on August 10, 2004. Appellants also provide the appeal fee. Please debit any underpayments, or credit any overpayments, to firm deposit account no. 08-1641.

I. Real Party in Interest

The captioned application is owned by Baxter International Inc. of Deerfield, Illinois and Baxter Healthcare S.A. of Zurich, Switzerland.

II. Related Appeals and Interferences

Appellants are unaware of any appeals, interferences or judicial proceedings which may be related to, directly affect or have a bearing on the Board's decision in the pending appeal.

III. Status of Claims

Claims 1-12 have been canceled without prejudice or disclaimer. Claims 13-17 stand finally rejected and are under appeal.

IV. Status of Amendments

Following the final rejection of December 17, 2003, Appellants filed an amendment after final rejection on May 6, 2004. On July 9, 2004, the examiner issued an advisory action, which entered the amendment after final rejection and resulted in the withdrawal of two indefiniteness rejections and an objection to the drawings. On August 10, 2004, Appellants filed a request for reconsideration, which was accompanied by three abstracts as evidence. The claims were not amended again. On November 12, 2004, the

examiner issued a second advisory action, which entered the August request for reconsideration, but did not result in an allowance of any claims.

V. Summary of Claimed Subject Matter

Appellants provide a concise explanation of the claimed invention for the Board. This explanation is illustrative only, and does not limit the claimed invention in any way. In the event of conflict, the captioned application controls over this explanation.

The claimed invention pertains to methods of producing pertussis toxins by cultivating *Bordetella pertussis* bacteria that lack cysteine desulfase activity and isolating the toxin from the media (claim 13). See the specification at paragraphs 0040-41 on pages 13-14. One approach to making such bacteria is to make so-called knock-out mutants of the cysteine desulfase gene (claim 14 and 15), which eliminate cysteine desulfase at the transcriptional level. See specification at page 26, paragraph 0065. That is, the gene is interrupted, thereby preventing formation of intact messenger RNA ("mRNA") that encodes the cysteine desulfase enzyme.

Cysteine desulfase gene sequences are disclosed at Figures 7A-7G of the specification. Techniques for cloning this gene, including the disclosure of appropriate primers, are set forth in the specification at page 22, paragraph 58. Methodologies for making cysteine sulfase knock-out mutants are set forth at page 13, paragraph 0040 to

page 15, paragraph 0043 of the specification. These methods include the use of homologous recombination to direct insertion of DNA into the cysteine desulfase gene in order to interrupt the sequence of the gene. Additionally, appellants have deposited a *Bordetella pertussis* knock out mutant, BP536pWY. See specification at page 23, paragraph 60.

Another approach to eliminating cysteine desulfase activity (claims 16 and 17) is through the use of anti-sense oligonucleotides, such as RNA, which operate at the translational level. See specification at pages 15-16, paragraph 0044. Sequences that are complementary to the cysteine desulfase gene sequences of Figures 7A-7G, such as SEQ ID NOS: 1 and 2, can be placed in the bacterial growth media, where they will be taken up by the bacteria and bind to cysteine desulfase mRNA therein, and thereby interfere with translation that would otherwise lead to production of the active cysteine desulfase enzyme.

Ultimately, reduction and/or elimination of the cysteine desulfase activity means that sulfate production is reduced. Sulfate is an inhibitor of pertussis toxin production, and therefore reduction in production of the inhibitor (sulfate) through the knock-out and anti-sense approaches leads to greater production of the pertussis toxin. See specification at page 3, paragraphs 0009-0014 and page 13, paragraph 0041.



VI. Grounds of Rejection to be Reviewed on Appeal

The examiner rejected the claims on written description grounds. Specifically, the examiner stated that the "claims encompass a genus of cysteine desulfonase genes." The examiner then stated that "[t]here is substantial variability among the species of cysteine desulfonase genes encompassed within the scope of the claims." See page 2 of the Advisory Action mailed July 9, 2004.

VII. Argument

A. The claimed invention is supported by the written description

The Federal Circuit has held that the written description requirement of 35 USC § 112 requires an applicant to provide sufficient description showing that the applicant possessed the claimed invention at the time of filing. *The Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1405 (Fed. Cir. 1997); *Fiers v. Revel*, 984 F.2d 1164, 1170, 25 USPQ2d 1601, 1605 (Fed Cir. 1993). The purpose of the written description requirement is to show that "the inventor invented the claimed invention." *The Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1565, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), *quoting from Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Written description is a question of fact. *See Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991).

The claims and the rejections are discussed with these principles in mind, and should each be considered on the merits. The claims are reproduced below and in the claim appendix.

1. Claim 13

Claim 13 recited methods for producing a pertussis toxin. Claims 13 permits interference of cysteine desulfonase at the transcriptional and translational levels. Claim 13 is as follows:

13. A method of producing pertussis toxin, comprising cultivating *Bordetella pertussis* bacteria that lack cysteine desulfonase activity; and isolating the toxin from the media.

Claim 13 covers (i) the insertion of DNA to interrupt the *Bordetella pertussis* cysteine desulfonase gene and (ii) the use of anti-sense sequences to interfere with the translation of cysteine desulfonase mRNA to form cysteine desulfonase protein.

As stated above, the examiner contends that the claims covers "a genus of cysteine desulfonase genes." Although a genus of cysteine desulfonase genes may exist in all of

the bacteria found in nature, the claimed method concerns "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity." Accordingly, it is cysteine desulfonase activity in *Bordetella pertussis* that is quenched. Therefore, cysteine desulfonase activities that may be found in other bacteria ranging from *Escherichia* to *Vibrio* to *Staphylococcus* etc. are of no moment because those bacteria are not implicated in the method.

The claimed method is directed to the production of pertussis toxin from *Bordetella pertussis*, and appellants' specification sets for the sequence of the *Bordetella pertussis* cysteine desulfonase gene. As explained above, appellants' specification discloses methodologies for eliminating the activity of the protein product of the cysteine desulfonase gene. Even without appellants' disclosure of the cysteine desulfonase sequence, the skilled person following appellants' teachings to use a "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity" to produce pertussis toxin could use transposon- and irradiation-based methodologies to eliminate cysteine desulfonase activity in *Bordetella pertussis* bacteria. See the Hofreuter, Zhou and Mosqueda abstracts provided in the Request for Reconsideration after Final Rejection dated August 10, 2004 and entered by the Advisory Action dated November 12, 2004 (abstracts reproduced in the evidence appendix). Appellants' specification, however, provides the cysteine desulfonase gene sequence and disclosure of knock-out and anti-sense approaches, which facilitates attainment of the recited "*Bordetella pertussis* bacteria that lack cysteine

desulfinate activity." It is that bacteria which is used to make the pertussis toxin according to appellants' claims.

In view of the above explanation, appellants submit that it is clear that appellants' possessed and disclosed the invention that is claimed. The existence of other bacteria with other genes do not detract from this possession and disclosure. Only *Bordetella pertussis* bacteria produce the pertussis toxin, and applicants have provided all of the information needed to practice the claimed method of producing pertussis toxin using "*Bordetella pertussis* bacteria that lack cysteine desulfinate activity." Appellants therefore request reversal of the rejection.

2. Claims 14 and 15

These claims are both dependent on claim 13, and are reproduced below:

14. The method according to claim 13, wherein the *Bordetella pertussis* bacteria are mutants having an DNA sequence integrated into a *Bordetella pertussis* cysteine desulfinate gene.

15. The method according to claim 14, wherein the mutant is strain BP536pWY.

Claim 14 requires the integration of a DNA sequence into a *Bordetella pertussis* cysteine desulfinate gene, and therefore functions at the transcriptional level. Again,

appellants' specification discloses the sequences of the cysteine desulfase gene (Figures 7A-7G) and methodologies for integrating DNA into the cysteine desulfase gene (paragraphs 0040-0041). Appellants actually performed this when strain BP536pWY was created and later deposited at the American Type Culture Collection. See paragraph 0060 and claim 15. Given this disclosure that establishes poession, the examiner's rejection is unsustainable and should be reversed.

3. Claims 16 and 17

Claims 16 and 17 are dependent on claim 13, and concern the anti-sense approach, which functions at the translational level. The claims are reproduced below:

16. The method according to claim 13, wherein the *Bordetella pertussis* bacteria are cultivated in the presence of cysteine desulfase anti-sense sequences.

17. The method according to claim 16, wherein the anti-sense sequences are 8 to 15 bases in length and complementary to a nucleotide sequence set forth in SEQ ID NOS: 8-12.

Approaches for making anti-sense oligonucleotides are set forth at paragraphs 0044-0045 on pages 15-16 of the specification. The anti-sense oligonucleotides are based upon the sequences set forth in appellants' Figures 7A-7G, and exemplary

oligonucleotide sequences are set forth in SEQ ID NOS: 1 and 2. In view of appellants' teachings, the skilled person can routinely synthesize additional anti-sense oligonucleotides in order to achieve "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity" for use in the production of pertussis toxin.

As was true for the other claims, the examiner's concerns about gene sequences in other bacteria have no applicability to appellants' claims. The skilled person who seeks to produce pertussis toxin will choose anti-sense sequences needed to inhibit the effect of the *Bordetella pertussis* cysteine desulfonase gene, and such a person would turn to Figures 7A-7G for the sequence information allowing the design of such anti-sense oligonucleotides. There would be no need to turn to other bacteria types given appellants' disclosure of the *Bordetella pertussis* cysteine desulfonase gene.

The examiner has provided no evidence or argument showing that the skilled person would be concerned with or desire genes from other bacteria types. Rather, appellants submit that the skilled person would utilize the sequences in Figures 7A-7G, and would be of the view that Appellants possessed an anti-sense approach to achieve a "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity." Appellants therefore have provided a written description that is commensurate with what is claimed.

B. Elucidation of structure is not a prerequisite of the claimed method

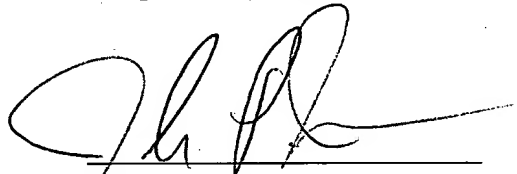
Each of the claims concern methods of using a "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity." The claims are not directed to compositions of matter *per se*. Accordingly, the examiner's concerns about structure are not understood.

Again, the claimed invention is directed to methods of producing pertussis toxin using "*Bordetella pertussis* bacteria that lack cysteine desulfonase activity" -- an activity which can be quenched at the transcriptional and translational levels. Once the appropriate integrating or anti-sense sequences are designed in view of the sequences of Figures 7A-7G, the skilled person will achieve the recited *Bordetella pertussis* bacteria after employing the disclosed techniques, cited above. Additional structural elucidation is unnecessary because all of the structures of the recited elements are known in achieving and using the recited *Bordetella pertussis* bacteria.

VIII. Requested Relief

Appellants submit that the rejections should be reversed. Appellants respectfully request that the rejections be reversed and that the application be returned to the examiner with instructions for an allowance.

Respectfully submitted,

  
\_\_\_\_\_  
John P. Isacson  
Reg. No. 33,715

December 14, 2004

Heller Ehrman White & McAuliffe LLP  
1666 K Street, N.W., Suite 300  
Washington, D.C. 20006  
Telephone: (202) 912-2000  
Facsimile: (202) 912-2020  
Customer No. 26633

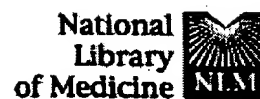


Claim Appendix for U.S. Serial No. 09/825,769

13. A method of producing pertussis toxin, comprising cultivating *Bordetella pertussis* bacteria that lack cysteine desulfase activity; and isolating the toxin from the media.
14. The method according to claim 13, wherein the *Bordetella pertussis* bacteria are mutants having an DNA sequence integrated into a *Bordetella pertussis* cysteine desulfase gene.
15. The method according to claim 14, wherein the mutant is strain BP536pWY.
16. The method according to claim 13, wherein the *Bordetella pertussis* bacteria are cultivated in the presence of cysteine desulfase anti-sense sequences.
17. The method according to claim 16, wherein the anti-sense sequences are 8 to 15 bases in length and complementary to a nucleotide sequence set forth in SEQ ID NOS: 8-12.

Evidence Appendix for U.S. Serial No. 09/825,769

Contains evidence entered by the Advisory Action dated November 12, 2004



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## Natural competence for DNA transformation in *Helicobacter pylori*: identification and genetic characterization of the comB locus.

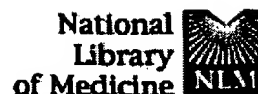
Hofreuter D, Odenbreit S, Henke G, Haas R.

Max-Planck-Institut für Biologie, Abteilung, Infektionsbiologie, Tübingen, Germany.

The gram-negative bacterial pathogen *Helicobacter pylori*, an important aetiological agent of gastroduodenal disease in humans, belongs to a group of bacterial species displaying competence for genetic transformation. Here, we describe the comB gene locus of *H. pylori* involved in DNA transformation competence. It consists of a cluster of four tandemly arranged genes with partially overlapping open reading frames, orf2, comB1, comB2 and comB3, constituting a single transcriptional unit. Orf2 encodes a 37-amino-acid peptide carrying a signal sequence, whereas comB1, comB2 and comB3 produce 29 kDa, 38 kDa and 42 kDa proteins, respectively, as demonstrated by immunoblotting with specific antisera. For Orf2 and ComB1, no homologous proteins were identified in the database. For ComB3, the best homologies were found with TraS/TraB from the *Pseudomonas aeruginosa* conjugative plasmid RP1 and TrbI of plasmid RP4, VirB10 from the Ti plasmid of *Agrobacterium tumefaciens* and PtlG, a protein involved in secretion of pertussis toxin of *Bordetella pertussis*. Defined transposon knock-out mutants in individual comB genes resulted in transformation-defective phenotypes ranging from a 90% reduction to a complete loss of the natural transformation efficiency. The comB2 and comB3 genes show homology to HP0528 and HP0527, respectively, located on the cagII pathogenicity island of *H. pylori* strain 26695.

PMID: 9663688 [PubMed - indexed for MEDLINE]

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## Multiple defects of cell cycle checkpoints in U937-ASPI3K, an U937 cell mutant stably expressing anti-sense ATM gene cDNA.

Zhou J, Liu W, Sun L, Sun H, Tang Y.

Department of Hematology, Tongji Hospital, Tongji Medical University, Wuhan 430030.

(Ataxia-telangiectasia mutated gene (ATM) functions in control of cell cycle checkpoints in responding to DNA damage and protects cells from undergoing apoptosis. Knock-out within tumor cells of endogenous ATM will achieve therapeutic benefits and enable a better understanding of the decisive mechanisms of cell death or survival in response to DNA damaging agents.) In present paper, we sought to characterize the cell cycle checkpoint profiles in U937-ASPI3K, a U937 cell mutant that was previously established with endogenous ATM knock-out phenotype. Synchronized U937-ASPI3K was exposed to 137Cs irradiation, G1, S, G2/M cell cycle checkpoint profiles were evaluated by determining cell cycle kinetics, p53/p21 protein, cyclin dependent kinase 2 (CDK2) and p34CDC2 kinase activity in response to irradiation. U937-ASPI3K exhibited multiple defects in cell cycle checkpoint as defined by failing to arrest cells upon irradiation. The accumulation of cellular p53/p21 protein and inhibition of CDK kinase was also abolished in U937-ASPI3K. It was concluded that the stable expression of anti-sense PI3K cDNA fragment completely abolished multiple cell cycle checkpoints in U937-ASPI3K, and hence U937-ASPI3K with an AT-like phenotype could serve as a valuable model system for investigating the signal transduction pathway in responding to DNA damaging-based cancer therapy.

PMID: 12840909 [PubMed - indexed for MEDLINE]

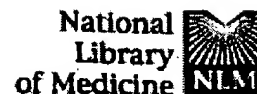
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### **A set of genes encoding a second toluene efflux system in *Pseudomonas putida* DOT-T1E is linked to the *tod* genes for toluene metabolism.**

**Mosqueda G, Ramos JL.**

Department of Biochemistry and Molecular Biology of Plants, Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Cientificas, E-18008 Granada, Spain.

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Sequence analysis in *Pseudomonas putida* DOT-T1E revealed a second toluene efflux system for toluene metabolism encoded by the *ttgDEF* genes, which are adjacent to the *tod* genes. The *ttgDEF* genes were expressed in response to the presence of aromatic hydrocarbons such as toluene and styrene in the culture medium. To characterize the contribution of the *TtgDE* system to toluene tolerance in *P. putida*, site-directed mutagenesis was used to knock out the gene in the wild-type DOT-T1E strain and in a mutant derivative, DOT-T1E-18. This mutant carried a Tn5 insertion in the *ttgABC* gene cluster, which encodes a toluene efflux pump that is synthesized constitutively. For site-directed mutagenesis, a cassette to knock out the *ttgD* gene and encoding resistance to tellurite was constructed in vitro and transferred to the corresponding host chromosome via the suicide plasmid pKNG101. Successful replacement of the wild-type sequences with the mutant cassette was confirmed by Southern hybridization. A single *ttgD* mutant, DOT-T1E-1, and a double mutant with knock outs in the *ttgD* and *ttgA* genes, DOT-T1E-82, were obtained and characterized for toluene tolerance. This was assayed by the sudden addition of toluene (0.3% [vol/vol]) to the liquid culture medium of cells growing on Luria-Bertani (LB medium (noninduced) or on LB medium with toluene supplied via the gas phase (induced). Induced cells of the single *ttgD* mutant were more sensitive to sudden toluene shock than were the wild-type cells; however, noninduced wild-type and *ttgD* mutant cells were equally tolerant to toluene shock. Noninduced cells of the double DOT-T1E-82 mutant did not survive upon sudden toluene shock; however, they still remained viable upon sudden toluene shock if they had been previously induced. These results are discussed in the context of the use of multiple efflux pumps involved in solvent tolerance in *P. putida* DOT-T1E.

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